Research Paper

Effect of phytosterol structure on thermal polymerization of heated soybean oil

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This study determined the effect of phytosterol structure, including the degree of unsaturation and the presence of an ethylidene group in the side chain, on the thermal polymerization of heated soybean oil. Indigenous tocopherols and phytosterols were removed from soybean oil by molecular distillation. Pure phytosterols were added back to the stripped soybean oil at concentrations of 0.5, 1.0, and 5 mg/g oil (0.05, 0.1, and 0.5 wt-%). These oils were heated at 180 °C over a period of 8 h, and triacylglycerol dimers and polymers, fatty acid composition, and residual phytosterol content were determined. None of the phytosterols prevented triacylglycerol dimer and polymer formation when used at 0.5 mg/g; however, phytosterols with two or more double bonds, regardless of the presence of an ethylidene group in the side chain, provided slight protection when added at 1 mg/g. Ergosterol addition at 5 mg/g reduced polymer formation by 16–20% compared to the control oil, but at this level none of the other phytosterols provided protection of any practical significance. Thus, under the conditions used for this heating study, the degree of phytosterol unsaturation was more important for its anti-polymerization activity than the presence of an ethylidene group.

Keywords: Ethylidene side chain / Fucosterol / Phytosterols / Thermal polymerization / Vegetable oils

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1 Introduction

Deep-fat frying is one of the most common uses for edible vegetable oils in the food industry. In 2006, about 7.8 billion lbs of fats and oils were used for baking and frying in the USA alone [1], which accounts for around 30% of total U.S. fat and oil consumption. Oil used for deep-fat frying is subjected to high temperatures, moisture from foods, and migration of other compounds from foods that could act as pro-oxidants. Vegetable oils high in polyunsaturated fatty acids (PUFA), such as regular soybean and sunflower oil, are considered unsuitable for commercial deep-fat frying. During frying, the PUFA are highly susceptible to oxidative damage, resulting in off-flavors and -odors, smoking and foaming of the frying oil, and production of oxidized and polymerized lipids that may be detrimental to human health [2]. Hydrogenation is commonly used to reduce the PUFA in these oils, thereby increasing the oil stability for frying. However, hydrogenating oils can cause the formation of fatty acids with *trans* double bonds [3], and consumption of *trans* fatty acids is believed to increase the risk of coronary heart disease [4]. Thus, with increasing knowledge of the effects of *trans* fatty acids on health, recent legislation requiring labeling of *trans* fatty acids in foods, as well as bans in certain U.S. cities on artificially produced *trans* fatty acids in restaurant foods, hydrogenation is no longer the most desirable solution. Antioxidants such as *tert*-butyl hydroquinone (TBHQ) and the anti-foaming agent dimethylsilicone are often added to oils to increase their fry-life; however, many antioxidants are less effective at frying temperatures because they quickly degrade or may volatilize [2]. Several varieties of vegetable oils with modified fatty acid composition, such as low-linolenic soybean oil or mid- and high-oleic sunflower oil and high-oleic or low-linolenic canola oil, have been introduced as more stable alternatives without

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the need for hydrogenation. Vegetable oils with modified fatty acid composition are produced through genetic modification, traditional plant breeding, blending of polyunsaturated oils with more saturated oils, or interesterification [5]. While fatty acid composition is probably the most important factor in determining oil stability to frying, the composition of minor components such as tocopherols is also an important determinant [6]. Thus, there is increasing interest in the role that natural minor oil constituents such as tocopherols and phytosterols have on stability to frying.

Phytosterols are triterpene compounds, similar in structure to cholesterol, that are found ubiquitously in plants as well as yeast, mold, and other fungi [1]. Phytosterols are the most dominant class of compounds in the unsaponifiable material of vegetable oils [7]. The three most abundant phytosterols found in commodity vegetable oils include β-sitosterol, campesterol, and stigmasterol. Several other sterols, such as Δ5- and Δ7-avenasterol as well as brassicasterol, are also common in vegetable oils, but are usually found in lower concentrations. Phytosterols, such as Δ5-avenasterol, its isomer fucosterol, as well as vernosterol and citrostadienol, have been found in several studies to protect oils subjected to frying temperatures from oxidative degradation, while other, more abundant phytosterols have been shown to have no activity [8–11]. For example, Sims *et al.* [8] reported slight protective activity of fucosterol, moderate activity of Δ7-avenasterol, and high activity of vernosterol in protecting heated safflower oil from decreasing in iodine value (IV). They also tested ergosterol, lanosterol, β-sitosterol, stigmasterol, and cholesterol and found them all to be ineffective. Gordon and Magos [9] also reported protection from reduction in IV by fucosterol and Δ5-avenasterol added to technical triolein heated to 180 °C. The protective phytosterols have in common an ethylidene group in their side chain (Fig. 1). Gordon and Magos [9] hypothesized that lipid free radicals react quickly with the unhindered allylic methyl hydrogens in the ethylidene side chain to form stable tertiary free radicals that are slow to react further, thus interrupting the chain of autooxidation like a classic chain-breaking antioxidant. There is scarce data, such as isolation of phytosterol side chain oxidation products, to support this hypothesized mechanism. Some non-steroidal compounds with an ethylidene group, such as undecylenic acid, linalyl acetate, and linalyl oleate, were shown to also have protective activity in oils heated to high temperatures [10, 12]. However, Onal-Ulusoy [12] also found that terpenyl oleates, both with and without an ethylidene structure, had antioxidant activity; so the activity of linalyl oleate could not be solely attributed to the ethylidene group. In the literature, phytosterols with an ethylidene group have been referred to as "antioxidants" and "polymerization inhibitors", despite the fact that in these studies oxygen consumption was not followed, the mechanism(s) for phytosterol activity were not determined, and in most cases, the degree of triacylglycerol polymerization was not determined.

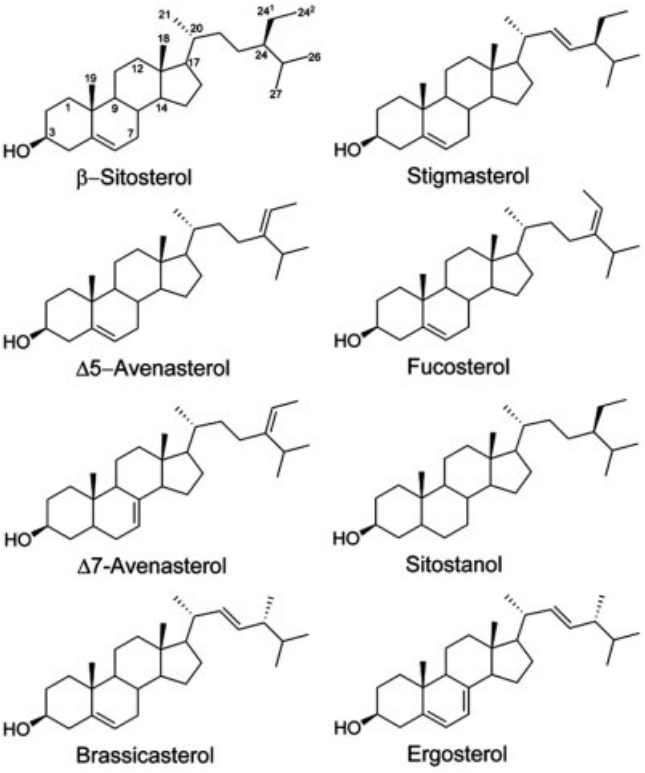

Figure 1. Structures and common names for the phytosterols used in this study as well as several other phytosterols (Δ5- and Δ7-avenasterol) with ethylidene structures in the side chains. International Union of Pure and Applied Chemistry-International Union of Biochemistry (IUPAC-IUB, 1989) numbering is shown for β-sitosterol.

We recently reported that a commercial mixture of pure phytosterols reduced thermal polymerization of soybean oil triacylglycerols, but not high-oleic sunflower oil triacylglycerols, when added at levels ranging from 1 to 2.5% [13]. Since the mixture was mainly composed of β-sitosterol, stigmasterol, and campesterol, with only small amounts of Δ5-avenasterol, it did not appear that the anti-polymerization activity was conferred by the Δ5-avenasterol. Rather, it seemed to be an effect of the higher total phytosterol concentration, and we hypothesized that at the higher concentrations some of the phytosterols may have reacted with radicals and peroxides of linoleic and linolenic acids, and thus terminated their further reaction. In order to further determine the anti-polymerization activity of phytosterols as related to their structure and number of double bonds, the goal of the present study was to compare the effect of phytosterols with zero to three double bonds, or an ethylidene group in the side chain, on thermal polymerization of soybean oil triacylglycerols. Pure sitostanol, sitosterol, stigmasterol, fucosterol, brassicasterol, and ergosterol (Fig. 1) were added to soybean oil that had its native tocopherols and phytosterols removed [stripped soybean oil

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(SSBO)]. The treated oils were heated for 8 h at 180 °C. The effect of these phytosterols on polymerized triacylglycerol (PTAG) formation and loss of PUFA was investigated.

2 Materials and methods

2.1 Materials

Hexane-extracted, alkali-refined, bleached and deodorized soybean oil (SBO) was obtained from a commercial processor. Stigmasterol and 5α-cholestane were purchased from Matreya, Inc. (Pleasant Gap, PA, USA); campesterol, fucosterol, and brassicasterol were purchased from Steraloids (Newport, RI, USA); β-sitosterol, ergosterol, and sitostanol were from Sigma-Aldrich (St. Louis, MO, USA). Each phytosterol was of ≥97% purity. All other chemicals and solvents were obtained from Sigma-Aldrich, unless otherwise stated, and were of ACS grade or HPLC grade for solvents.

2.2 Molecular distillation

Tocopherols and most of the phytosterols were removed from the soybean oil using a model ICL-04A short-path thin-film evaporator unit (Incon Processing, Batavia, IL, USA) attached to a high-vacuum pump and a diffusion pump. The oil was first de-gassed by passing through the evaporator at ~2–3 mL/min under vacuum of 100 mTorr and an evaporator temperature of 250 °C. The de-gassed oil was passed through the evaporator a second time at the same rate and temperature, but under increased vacuum of 1 mTorr. The SSBO was examined as described below for residual tocopherols, phytosterols, fatty acid composition, and peroxide value (PV). The stripped oil was placed in dark-glass screw-cap bottles, sealed with argon in the headspace, and kept frozen at –80 °C until used.

2.3 Phytosterol addition to oils

The phytosterols were weighed into tared test tubes and dissolved in an appropriate amount of chloroform. The exact concentration of each phytosterol in the stock solutions was verified after derivatization and GC analysis as described below. Aliquots of each phytosterol stock were pipetted into a foil-covered vial, and the solvent was removed under a gentle stream of nitrogen. SSBO was added to the vials to achieve concentrations of 0.5, 1.0, or 5.0 mg/g for each phytosterol. Typically, the oils with added phytosterols were made in 6-g batches. The phytosterols were dissolved in the oil by stirring and heating at 45 °C on a temperature-controlled hotplate for 10 min. A vial containing SSBO without added phytosterols was heated and stirred in the same manner. The contents of the vials were then capped with argon in the atmosphere, and frozen at –20 °C until used for the heating study (typically overnight).

2.4 Heating study

The oils were thawed at room temperature and aliquots (1 g) from each treatment were weighed into four, 13 × 100 mm open-mouth borosilicate tubes and placed in a test tube rack that was immersed in a circulating hot oil bath maintained at 180 ± 1 °C. The remaining oil was reserved frozen (–20 °C) for analysis. Two tubes per treatment were removed after 4 and 8 h. After cooling for 5 min, the oil was transferred to vials, covered with argon in the headspace and frozen until analyzed.

2.5 Oil analysis

2.5.1 Fatty acid composition

Fatty acid methyl esters (FAME) were prepared using the method of Ichihara et al. [14]. FAME were separated using a Varian (Palo Alto, CA, USA) 8400 GC equipped with an FID, an on-board integrator, and a Supelco (Bellefonte, PA, USA) SP2380 capillary column (30 m × 0.25 mm i.d., 0.20 μm film thickness). Carrier gas was He at 1 mL/min flow rate. The oven temperature was initially held at 150 °C for 15 min, then increased to 210 °C at 2 °C/min, followed by an increase to 220 °C at 50 °C/min. The injector and detector temperatures were set at 240 and 270 °C, respectively. FAME were identified by comparison of retention times with known standards. For the purpose of comparing our results with other reported results, the fatty acid profile was used to determine the calculated IV of the oils using the AOCS recommended practice Cd 1c-85 [15].

2.5.2 Peroxide value

PV of the oils were determined using the method of Shantha and Decker [16], except that it was scaled down and modified as described by Hu *et al.* [17]. Briefly, 10-mg samples were weighed into test tubes and dissolved by vortex in 3 mL methanol/butanol (2 : 1 vol/vol). Ammonium thiocyanate (15 μL of 3.94 M solution) was added and mixed for 3–4 s by vortex, followed by 15 μL ferrous chloride (0.018 M) solution. After 20 min of incubation at room temperature, the absorbance at 510 nm (A_{510}) was measured against a blank containing all of the reagents except for the sample, using a Shimadzu model UV-120-02 spectrophotometer (Columbia, MD, USA). PV were calculated from a standard curve of ferric chloride as described by Shantha and Decker [16]. PV are expressed as milliequivalents (meq) of peroxide per kilogram oil.

2.5.3 Triacylglycerol dimers and polymers

Portions of the oil were dissolved in tetrahydrofuran to a concentration of ~50 mg/mL. Total triacylglycerol dimers, trimers, tetramers, and higher-molecular-weight (Mw) poly-

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mers were analyzed by high-performance size-exclusion chromatography (HPSEC). The HPSEC system consisted of a Shimadzu (Columbia, MD, USA) LC20AT pump equipped with membrane degasser and an autosampler. Injected samples were separated into mono-, di-, and triglycerides as well as triacylglycerol dimers, trimers, tetramers, and higher-Mw polymers on three PLgel Mixed E columns (3 μm, 100 Å pore size, 300 × 7.5 mm; Polymer Labs, Amherst, MA, USA). Mobile phase was tetrahydrofuran (0.8 mL/min). Peaks were detected with an evaporative light scattering detector operated at a temperature of 40 °C with the nebulizer gas (ultra-pure N_2) pressure set to 2.5 bar and the gain set at 4. HPSEC control, data collection and analysis were performed by Shimadzu EZStart Chromatography Software Version 7.3. Each sample was analyzed in duplicate.

2.5.4 Tocopherol analysis

Oil samples were diluted in hexane to a concentration of 50–100 mg/mL and filtered through 0.45-μm centrifugal filters (National Scientific, Rockwood, TN, USA) for high performance liquid chromatography (HPLC) analysis. HPLC analysis was performed according to the AOCS official method Ce 8-89 [18]. The HPLC system consisted of a Varian ProStar pump, an autosampler, and a fluorescence detector. The mobile phase consisted of hexane/2-propanol (99.5 : 0.5 vol/vol, made fresh daily) pumped at 1 mL/min. Samples were injected by autosampler using the full-loop option (100 μL), and tocopherols were separated using an Inertsil (Varian) silica column(5 μm, 150 Å, 250 mm × 4.6 mm i.d.). Tocopherol peaks were identified by retention times of known standards. A mixture of α-, β-, γ-, and δ-tocopherol standards was injected on each day of analysis to verify HPLC performance. Tocopherols were quantified using external standard curves.

2.5.5 Phytosterol analysis

Oil samples (25–50 mg) were saponified and derivatized for phytosterol analysis as described by Dutta *et al.* [19]. Samples were injected by autosampler into a Varian 3800 GC equipped with an FID and a Supelco (Bellefonte, PA, USA) SPB™-1701 (30 m × 0.25 mm × 0.25 μm) capillary column. Helium was used as carrier gas, with a 1 : 50 injector split. The injector temperature was 270 °C, and the detector temperature was 290 °C. The column oven initial temperature was 250 °C for 0.5 min, increased at 10 °C/min to 270 °C and held for 27 min, then increased at 10 °C/min to 280 °C and held for 3.5 min. GC control, data collection and integration were performed using Varian Star Chromatography Software Ver. 5.3. Phytosterols were identified by comparison of their retention times (relative to 5α-cholestane) with those of commercially available standards. Quantitation was carried out by the internal standard method.

2.6 Statistical analysis

The effects of phytosterol treatments on the weight percentage of linoleic and linolenic acids, the calculated IV, and triacylglycerol dimer and PTAG content at different heating times were compared by analysis of variance using Statistical Analysis System Version 9.1 (SAS Inc., Cary, NC, USA). The mean values for the dependent variables for each treatment (control SSBO and SSBO with added phytosterols) at each time point were compared using Tukey's multiple comparisons test, where $p < 0.05$ was deemed statistically different.

3 Results

3.1 Initial oil analysis

We first determined the effect of molecular distillation on the FAME, tocopherol, and phytosterol composition of the SSBO, and on the PV. The FAME composition of the soybean oil did not change after molecular distillation (Table 1). However, 99.5 and 87% of the tocopherols and phytosterols, respectively, were removed. Analysis of the SSBO by TLC revealed that the remaining phytosterols were acyl esters, which have a higher Mw than free phytosterols and thus were not as efficiently removed by molecular distillation (data not shown). Based on the concentration of phytosterols in the

Table 1. Chemical characteristics of soybean oil before and after molecular distillation.

	SBO	SSBO
Fatty acid composition [%]		
16:0	9.8	9.8
18:0	4.6	4.6
18:1	25.5	25.5
18:2	52.6	52.6
18:3	6.8	6.8
20:0	0.4	0.4
22:0	0.4	0.4
PV [meq peroxide/kg]	0.38	0.42
Tocopherol content [ppm]	973.13	4.6
Phytosterol content [mg/g]		
Brassicasterol	ND	ND
Campesterol	0.69	0.05
Campestanol	0.01	ND
Stigmasterol	0.59	0.02
Sitosterol	1.81	0.24
Sitostanol	0.05	0.01
Δ5-Avenasterol	0.09	0.03
Δ7-Stigmastenol	0.21	0.09
Total	3.45	0.44

SBO, soybean oil before molecular distillation; SSBO, stripped soybean oil (after molecular distillation); ND, not detected.

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SSBO (0.44 mg/g), the addition of 0.5, 1.0, and 5.0 mg/g represented an approximate 2-, 4-, and 12-fold increase in the total phytosterol content, respectively. The stripped oils were used as controls for all three experiments. A sample of the unstripped SBO was included in the last heating study for the purposes of comparison and to demonstrate the effect of minor component removal on heat stability.

3.2 Heating study results

3.2.1 Triacylglycerol polymers

Thermal polymerization is initiated by the formation of alkyl radicals which can form dimers, polymers, and even cyclic dimers and polymers by C-C, C-O-C, or C-O-O-C bonds [2]. Total PTAG (including dimers, trimers, tetramers, and higher-Mw polymers), is considered one of the best indicators of frying oil degradation, and many European countries mandate that a frying oil or fat be discarded when levels exceed 12–13% [20]. The formation of triacylglycerol dimers and the sum of all PTAG for the SSBO with added phytosterols are shown in Table 2. At the lowest concentration (0.5 mg/g), and after 4 h of heating, SSBO with added fucosterol and ergosterol were slightly but significantly ($p < 0.05$) lower in dimers and PTAG compared to SSBO alone. However, this protection was not evident after 8 h. At the 1.0-mg/g level, SSBO with added stigmasterol, fucosterol, and ergosterol were significantly ($p < 0.05$) lower in dimers and total PTAG after 8 h compared to SSBO alone. Neither sitostanol nor β-sitosterol had any impact on PTAG formation. Despite the fact that fucosterol has an ethylidene group in its side chain, there was no significant difference in protective effect between stigmasterol, ergosterol, and fucosterol. In the third heating study, at the 5-mg/g (0.5%) addition level, regular SBO (not stripped of tocopherols and phytosterols) was included for comparison to SSBO and SSBO with added phytosterols. In addition, brassicasterol, another phytosterol containing two double bonds, was included as another treatment to determine if it had similar activity to stigmasterol and fucosterol. However, the protective effect of stigmasterol and fucosterol did not increase at the higher concentration, and in fact, these compounds did not have any significant impact on dimer or PTAG formation when added to SSBO. SSBO with added brassicasterol was only slightly but significantly lower in dimers and PTAG compared to SSBO alone. On the other hand, ergosterol significantly lowered dimer and PTAG formation in SSBO at this concentration. Compared to SSBO alone, ergosterol decreased PTAG formation by 21.9 and 16.6% after 4 and 8 h, respectively. Compared to SSBO, regular SBO was 65.6 and 64.3% lower in PTAG content after 4 and 8 h, respectively. Thus, the protection by added ergosterol did not completely compensate for the antioxidant activity of the components removed by molecular distillation.

Table 2. Formation of triacylglycerol dimers and total polymers in SSBO with added phytosterols and heated at 180 °C.

Time [h]	Treatment	Phytosterol addition level					
		0.5 mg/g (0.05%)		1.0 mg/g (0.1%)		5.0 mg/g (0.5%)	
		Dimers [area%]	Total† [area%]	Dimers [area%]	Total [area%]	Dimers [area%]	Total [area%]
4	SBO	ND‡	ND	ND	ND	3.4 ± 0.1^{d}	3.7 ± 0.2^{d}
	SSBO	8.8 ± 0.1^{a}	11.2 ± 0.2^{a}	8.1 ± 0.5^{a}	10.1 ± 0.6^{a}	8.5 ± 0.1^{b}	10.7 ± 0.2^{b}
	+ Sitostanol	$8.5 \pm 0.2^{a,b,c}$	$10.7 \pm 0.4^{a,b}$	8.3 ± 0.2^{a}	10.4 ± 0.3^{a}	$8.6 \pm 0.2^{a,b}$	$10.9 \pm 0.3^{a,b}$
	+ β-Sitosterol	$8.8 \pm 0.2^{a,b}$	$11.1 \pm 0.2^{a,b}$	8.1 ± 0.4^{a}	10.0 ± 0.7^{a}	$8.6 \pm 0.1^{a,b}$	$10.9 \pm 0.1^{a,b}$
	+ Stigmasterol	$8.4 \pm 0.2^{b,c}$	$10.5 \pm 0.4^{a,b}$	8.4 ± 0.3^{a}	10.3 ± 0.7^{a}	$8.7 \pm 0.0^{a,b}$	$11.0 \pm 0.1^{a,b}$
	+ Fucosterol	8.3 ± 0.0^{c}	10.3 ± 0.1^{b}	8.3 ± 0.1^{a}	10.3 ± 0.1^{a}	8.8 ± 0.1^{a}	11.2 ± 0.2^{a}
	+ Brassicasterol	ND	ND	ND	ND	$8.6 \pm 0.4^{a,b}$	$10.9 \pm 0.6^{a,b}$
	+ Ergosterol	$8.4 \pm 0.3^{b,c}$	$10.5 \pm 0.4^{a,b}$	8.1 ± 0.2^{a}	10.0 ± 0.3^{a}	6.9 ± 0.0^{c}	8.3 ± 0.0^{c}
8	SBO	ND	ND	ND	ND	6.5 ± 0.1^{e}	7.6 ± 0.2^{d}
	SSBO	12.1 ± 0.2^{a}	18.6 ± 0.6^{a}	13.1 ± 0.1^{a}	21.3 ± 0.4^{a}	13.1 ± 0.0^{a}	21.3 ± 0.3^{a}
	+ Sitostanol	12.5 ± 0.2^{a}	19.7 ± 0.5^{a}	$12.9 \pm 0.2^{a,b}$	$21.0 \pm 0.4^{a,b}$	$12.9 \pm 0.1^{b,c}$	21.2 ± 0.2^{a}
	+ β-Sitosterol	12.2 ± 0.3^{a}	18.5 ± 0.7^{a}	$12.9 \pm 0.3^{a,b}$	$20.9 \pm 1.0^{a,b}$	$12.9 \pm 0.1^{b,c}$	21.2 ± 0.4^{a}
	+ Stigmasterol	12.1 ± 0.2^{a}	18.7 ± 0.5^{a}	12.7 ± 0.2^{c}	20.2 ± 0.7^{c}	$13.0 \pm 0.2^{a,b}$	$21.0 \pm 0.7^{a,b}$
	+ Fucosterol	12.5 ± 0.3^{a}	20.0 ± 1.0^{a}	$12.8 \pm 0.0^{b,c}$	$20.4 \pm 0.1^{b,c}$	$13.0 \pm 0.1^{a,b,c}$	21.2 ± 0.1^{a}
	+ Brassicasterol	ND	ND	ND	ND	12.8 ± 0.1^{c}	20.6 ± 0.2^{b}
	+ Ergosterol	12.4 ± 0.3^{a}	19.4 ± 0.6^{a}	12.6 ± 0.0^{c}	20.1 ± 0.1^{c}	11.4 ± 0.1^{d}	17.8 ± 0.3^{c}

† The sum (area%) of dimers, trimers, tetramers, and higher-Mw PTAG.
‡ ND: not determined. See Table 1 for abbreviations.
Within each time period and phytosterol addition level, treatments in columns with the same superscript letter are not significantly different from each other at $p < 0.05$. There were no significant treatment effects at 0 h.

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3.2.2 Changes in fatty acid composition

The fatty acid composition of the oils was followed during heating in the third experiment to determine the impact that phytosterol treatments had in protecting PUFA from destruction. As Table 1 shows, the original composition of the SSBO triacylglycerols included 52.6% linoleic acid and 6.8% linolenic acid. The content of linoleic acid in SSBO decreased to around 48% while linolenic acid decreased to around 5.2% by the end of the 8-h heating study. There was a very slight but noticeable trend of protection of linoleic and linolenic acids by phytosterols, especially ergosterol and fucosterol (Fig. 2), which is also depicted by a lower percentage of decrease in the calculated IV (Table 3). However, this slight protection was not statistically ($p < 0.05$) significant.

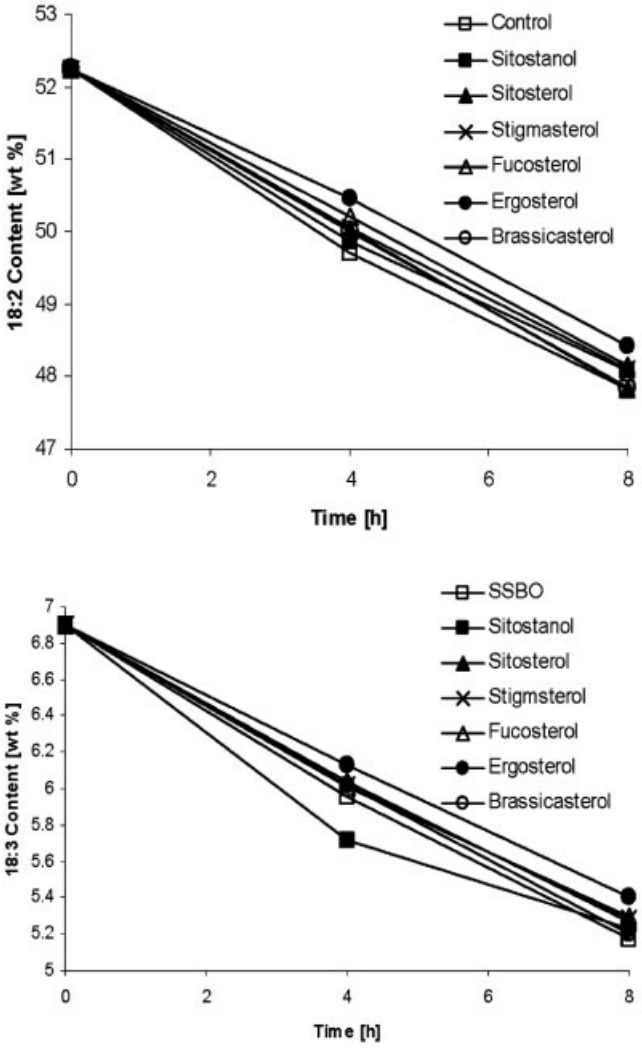

Figure 2. The reduction in linoleic (18:2) and linolenic (18:3) acids in SSBO and SSBO with added phytosterols at the 5-mg/g level, when heated at 180 °C.

3.2.3 Reduction in phytosterols

Phytosterols can undergo oxidation to form various oxidized sterol products including epoxy-, keto-, and hydroxy-sterols [21–23] as well as non-oxygenated sterol products including steradienes [21]. These reactions are accelerated at higher temperatures, and are also dependent on the degree of unsaturation of the phytosterol and on the lipid matrix. Soupas *et al.* [22] found that at 180 °C stigmasterol oxides formed more rapidly in a tripalmitin matrix than in a rapeseed oil matrix, but at lower temperatures (60–100 °C) stigmasterol oxides formed more rapidly in the rapeseed oil. It was concluded that at high temperatures the PUFA matrix was more rapidly oxidized and thus shielded the phytosterols from oxidation. By measuring the residual, intact phytosterols during heating, we can also get an indirect measurement of the amounts that have undergone oxidation and/or thermal destruction. This also may lend insight into their impact on polymer formation. In all three experiments, approximately 90% of the remaining native phytosterols in the control SSBO, composed mostly of β-sitosterol, remained intact after 8 h, while approximately 86.5% of the native phytosterols in the regular, unstripped SBO were intact after 8 h in the third experiment (Fig. 3A, B). Concentration was one factor determining the loss of phytosterols added to SSBO, *i.e.* more phytosterols as well as a higher percentage were destroyed at higher addition concentrations. For example, when sitostanol was added to SSBO at 0.5 mg/g, essentially all of it remained intact after 8 h of heating (Fig. 3A), whereas at the 5-mg/g addition level 11.6% of the sitostanol was destroyed (Fig. 3B). This is consistent with our previous findings that the thermal destruction of phytosterols was concentration dependent when mixtures of phytosterols were added to stripped soybean and sunflower oils [13]. Saturated sitostanol was more stable than the other phytosterols which had one to three double bonds, which is consistent with other studies showing an increase in the formation of sterol oxidation products with increasing unsaturation [22]. At the lowest concentration (0.5 mg/g), only 80% of intact fucosterol remained after 8 h compared to 87.4 and 90% of sitosterol and stigmasterol, respectively (Fig. 3A). As the concentration increased, the difference in stability between phytosterols with one or two double bonds seemed to decrease, but fucosterol was still slightly preferentially destroyed, because at 5 mg/g, 76.5% sitosterol, 73.7% stigmasterol, and 80.3% brassicasterol remained intact after 8 h compared to 70.7% fucosterol. In contrast to the relative stability of the other phytosterols, there was very little intact ergosterol remaining after 8 h in all three studies (Fig. 3). Ergosterol is structurally the same as brassicasterol, except for the additional double bond at C7 in the steroidal ring (Fig. 1), which results in two allylic tertiary carbon centers compared to two allylic secondary carbon centers in brassicasterol. Thus, the increase in

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Table 3. Comparison of the effects of phytosterols on oxidation and polymerization of heated oil in this study to selected results from other studies in the literature.

Reference	Phytosterol	Time (hr)	% ΔIV[†]	% Δ18:3[‡]	%PTAGs
This study	Control (stripped soybean oil)	8	7.1	25	21.3
	0.5% Fucosterol	8	6.5	23.3	21.2
	0.5% Ergosterol	8	5.6	21.7	17.8
9	Control (Safflower oil)	21	7.6	ND[§]	ND
	0.05% Fucosterol	21	6.2	ND	ND
	0.05% Δ7-Avenasterol	21	4.8	ND	ND
	0.05% Vernosterol	21	0.02	ND	ND
	Control (Safflower oil)	50	18.6	ND	ND
	0.05% Fucosterol	50	17.9	ND	ND
	0.05% Δ7-Avenasterol	50	13.8	ND	ND
	0.05% Vernosterol	50	11.0	ND	ND
10	Control (technical tri-olein)	25	10.0	ND	ND
	0.1% Δ5-Avenasterol	25	5.2	ND	ND
	0.1% Fucosterol	25	5.8	ND	ND
	Control (technical tri-olein)	50	18.6	ND	ND
	0.1% Δ5-Avenasterol	50	11.6	ND	ND
	0.1% Fucosterol	50	10.5	ND	ND
11	Control (soybean oil)	14	ND	20	ND
	0.02% Δ7-Avenasterol	14	ND	0	ND
	Control (soybean oil)	48	ND	60	ND
	0.02% Δ7-Avenasterol	48	ND	40	ND
12	Control (soybean oil)	7	ND	28.3	ND
	0.17% sterol fraction B	7	ND	27	ND
	Control (soybean oil)	35	ND	82.3	ND
	0.17% sterol fraction B	35	ND	74.9	ND
	Control (soybean oil) [††]	7	ND	26.9	ND
	0.25% sterol fraction B [††]	7	ND	5.8	ND
24	Control (purified high-oleic sunflower oil)	12	ND	ND	19.7
	0.1% Fucosterol	12	ND	ND	19.3
	Control (purified high-oleic sunflower oil)	48	ND	ND	52.7
	0.1% Fucosterol	48	ND	ND	52.8

[†]Percentage decrease in Iodine Value; [‡]Percent decrease in the weight percentage of linolenic acid; [§]ND: not determined; [#]Consisting of a mixture of β-sitosterol and Δ5-Avenasterol purified from oat lipids, the percentage of each component in the mixture was not reported; [††]This was a separate experiment from the other data shown from this reference

ergosterol destruction is most likely due not only to the greater number of double bonds but also to the reduced bond dissociation energies of the allylic tertiary carbon-hydrogen bonds in the ergosterol ring.

4 Discussion

The objective of this study was to determine the activity of phytosterols in preventing thermal polymerization of soybean oil triacylglycerols in relation to the degree of unsaturation or the presence of an ethylidene group in the side chain. Saturated sitostanol and monounsaturated β-sitosterol had no effect on dimer and PTAG formation at any of the studied concentrations. Stigmasterol and fucosterol, with two double bonds, and ergosterol, with three double bonds, all had a slight protective effect when added to SSBO at 0.1%. However, at 0.5%, stigmasterol and fucosterol had no protective effect and brassicasterol, which also has two double bonds, only had a slight protective effect. Only ergosterol conferred both a statistically significant as well as a practically appreciable decrease in PTAG formation when added to SSBO at 0.5%. Therefore, it appears that under the conditions that were used in this heating study, the presence of an ethylidene group in the side chain did not influence the anti-polymerization activity, and the number of double bonds and their location in the ring had a greater influence on the anti-polymerization activity.

In order for phytosterols to prevent the thermal polymerization of triaclyglycerols, they must be relatively reactive to free radical formation, thus quenching lipid radicals, and the

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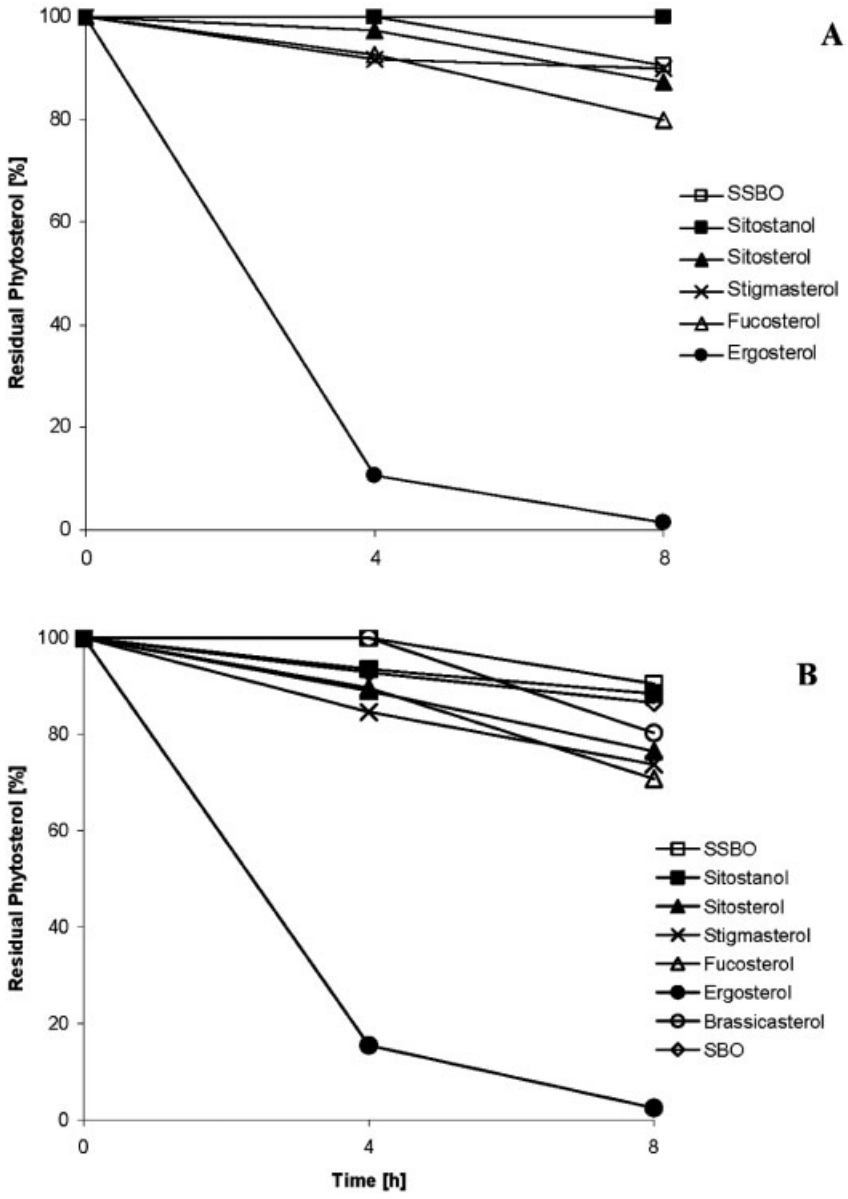

Figure 3. Percentage (of the initial amount) of phytosterols remaining in SBO, SSBO, and SSBO with added phytosterols, when added at 0.5 mg/g (A) and 5 mg/g (B) and heated at 180 °C.

free radicals formed must also be rather stable and slow to react further. Saturated sterols such as sitostanol are slow to form free radicals and undergo oxidation [22], explaining its relative inertness in this study. Several studies have shown that ring-unsaturated sterols such as sitosterol and stigmasterol are much more reactive than sitostanol [21, 22] due to the higher reactivity of the allylic secondary carbon centers. This would explain the increased loss of phytosterols with one or more double bonds compared to sitostanol in this study. The secondary free radicals formed in the ring of these phytosterols have only one resonance structure and are thus not very stable, which likely explains why they cannot effectively inhibit SSBO polymerization. There is a dearth of information in the literature on the formation of phytosterol side chain oxidation products. As mentioned, Gordon and Magos [9] hypothesized that the protons on the allylic primary carbon ($C24^2$) of ethylidene side chain phytosterols were less hindered, and that the free radical thus formed could then isomerize to a stable tertiary free radical at C_{24}. In this study, we found that fucosterol loss was slightly greater than that of stigmasterol and brassicasterol, indicating that the hydrogens on carbons allylic to the ethylidene group may have been more labile. However, fucosterol did not inhibit polymerization much more effectively than the other sterols, indicating that the radicals formed were not stable enough to interrupt the chain of oxidation. This may either be due to the slow isomerization of the primary radical to the tertiary radical or to the low number of resonance structures possible with this tertiary free radical.

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The lack of protective effect of fucosterol in this study is in contrast to several reports that sterols with an ethylidene group in the side chain, when added at concentrations ranging from 0.05 to 0.1%, protected oils that were heated to frying temperatures [8–11]. However, Lampi *et al.* [24] also reported that fucosterol (0.05–0.1%) was not effective in inhibiting polymerization of purified high-oleic sunflower oil triacylglycerols [24]. The contrasting results may be due to differences in testing methodology and the fatty acid composition of the oils used as a medium. These factors have varied considerably from study to study. In Table 3 we provide a comparison of our results, using 0.5% fucosterol and ergosterol in SSBO, to selected results obtained with phytosterols in several of the above-mentioned studies. Since different methods were used for determining the extent of oil degradation, this table allows a visual comparison of the reported activities and the extent of oil degradation obtained in these different studies, from which several observations can be made. Phytosterols with ethylidene groups in their side chain have been found to have protective activity in various oils, including safflower oil [8], technical triolein [9], and soybean oil [10, 11]. However, as mentioned previously, fucosterol (0.05–0.1%) was not effective in inhibiting polymerization of purified high-oleic sunflower oil triacylglycerols [24], which had a similar fatty acid composition to the technical triolein used by Gordon and Magos [9]. Thus, differences in oil fatty acid composition do not completely explain the contrasting results in the literature, since fucosterol has shown varying results in different studies with oils of similar fatty acid composition. The presence of tocopherols also does not seem to play a major role in the activity of these phytosterols, since protective activity has been demonstrated both in native oils as well as in purified triacylglycerols.

Factors such as surface-to-volume ratio and method of heating are both known to impact oxidation rates of heated oils, as well as the activity of antioxidants [25]. Neither the surface-to-volume ratios nor the beaker dimensions were typically given in the comparison studies, but based on the dimensions of standard 150- and 250-mL beakers, calculated surface-to-volume ratios likely would have been slightly lower than were used in the current study ($\sim$1.1 cm^{-1}) as well as in the study conducted by Lampi *et al.* [24] ($\sim$3.5 cm^{-1}). While a lower surface-volume ratio would be expected to result in decreased oxidation rates, heating with a hotplate was shown to increase oxidation rates compared to heating in an oven [25]. It appears by comparing either the percent decrease in IV or 18:3 in our study at the 8-h end-point to other studies at various end-points [8–11] that overall oxidation rates were faster in our study compared to some of the other studies. Based on the PTAG formation, degradation rates were similar in our study and the study on high-oleic sunflower oil [24]. The increased rate of oxidative degradation was likely due to the combination of the low volume of oil, the higher surface-to-volume ratios, and the removal of tocopherols. The increased rate of oxidation may at least partly explain the apparent lack of protective activity of fucosterol compared to other studies, due, as mentioned above, to the instability of the fucosterol radicals formed. Thus, much more information is needed on the mechanism for the activity of ethylidene side chain-containing phytosterols in order to fully understand why fucosterol was not very effective in preventing polymerization under the conditions of our study.

Sims *et al.* [8] reported that ergosterol was ineffective in inhibiting the decrease in IV of safflower oil heated to 180 °C, though they did not actually show the data. However, it was probably tested at a level of 0.05% (the level used for the other compounds tested in that study), while we found it was better at inhibiting polymerization at 0.1 and 0.5%. The rapid loss of ergosterol during this study confirms that the higher number of double bonds as well as the conjugation in the ring contributed to its instability. There are three possible tertiary allylic carbons as well as one possible secondary allylic carbon in the ergosterol rings, and free radicals formed at any of these locations would have several resonance structures to increase their stability. This likely explains the inhibition of polymerization seen at higher concentrations, where ergosterol was able to compete with PUFA for oxygen and lipid radicals. However, these are speculations at this point, and further research into the mechanism for anti-polymerization activity of ergosterol is warranted.

5 Conclusions

Phytosterols with two double bonds such as stigmasterol, fucosterol, and brassicasterol had only slight anti-polymerization activity in heated SSBO, but the ethylidene side chain did not appear to confer any increased activity for fucosterol under the conditions of our study. Ergosterol showed similar activity to the other phytosterols when added at 0.05 and 0.1%, but it significantly slowed PTAG formation when added to SSBO at 0.5%. Therefore, under the conditions of this study, the anti-polymerization activity appeared to be more dependent on the number and location of double bonds in the ring structure rather than on the presence of an ethylidene group in the phytosterol side chain. The mechanism for the activity of ergosterol as well as other phytosterols with three or more double bonds should be investigated further. In addition, the formation of ergosterol and other sterol oxidation products must be studied more thoroughly to determine their impact on human health before it could be considered beneficial to add them to edible oils.

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Conflict of interest statement

The authors have declared no conflict of interest.

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